# Higher evening metabolic responses contribute to diurnal variation of self-paced cycling performance

AUTHORS: Wajdi Souissi<sup>1</sup>, Omar Hammouda<sup>1,2</sup>, Achraf Ammar<sup>1,3</sup>, Mohamed Ayachi<sup>1</sup>, Yasmine Bardiaa<sup>4</sup>, Omayma Daoud<sup>4</sup>, Imen Ben Hassen<sup>2</sup>, Mohamed Souissi<sup>4</sup>, Tarak Driss<sup>1</sup>

<sup>1</sup> Interdisciplinary Laboratory in Neurosciences, Physiology and Psychology: Physical activity, Health and learning (LINP2), UFR STAPS, UPL, Paris Nanterre University, Nanterre, France

- <sup>2</sup> Research Laboratory, Molecular Bases of Human Pathology, UR19ES13, Faculty of Medicine, University of Sfax, Tunisia
- <sup>3</sup> Institute of Sport Science, Otto-von-Guericke University, Magdeburg, Germany
- <sup>4</sup> Research Laboratory "Education, Motricity, Sport and Health", High Institute of Sport and Physical Education, University of Sfax, Tunisia

ABSTRACT: This study examined the effect of time of day (TOD) on physical performance, and physiological and perceptual responses to a 10-km cycling time trial ( $TT_{10km}$ ). Twelve physically trained subjects (20.3  $\pm$  1.2 years, 74.3  $\pm$  7.4 kg, 179.7  $\pm$  5.5 cm) completed, in a randomized order, a TT<sub>10km</sub> in the morning and in the evening. Intra-aural temperature (IAT) was measured at rest and following the TT<sub>10km</sub>. Completion time, power output (PO), rating of perceived exertion (RPE), heart rate (HR), minute ventilation (VE), oxygen uptake (VO<sub>2</sub>), carbon dioxide production ( $\dot{V}CO_2$ ) and respiratory exchange ratio (RER) were assessed every km during the TT<sub>10km</sub>. Blood lactate concentration [La] and blood glucose concentration [Glu] were assessed before, during and immediately after the TT<sub>10km</sub>. Faster completion time ( $\Delta$  = 15.0s, p = 0.03) and higher IAT ( $\Delta$  = 0.33°C, p = 0.02 for pre-TT<sub>10km</sub>) were obtained in the evening compared to the morning with a significant correlation between  $\Delta$  completion time and  $\Delta$  IAT at post-TT<sub>10 km</sub> (r = -0.83, p = 0.04).  $\dot{V}O_2$ , [La] and [Glu] increased significantly during both test sessions (p < 0.001) with higher values in the evening compared to the morning (p = 0.015, p = 0.04, p = 0.01, respectively). However, the remaining parameters were found to be only affected by the TT<sub>10km</sub> (p < 0.001). The TT<sub>10km</sub> generates a higher  $\dot{V}O_2$  and higher [La] and [Glu] responses, contributing to a better cycling performance in the evening compared to the morning. The similar magnitude of the TOD effect on completion time and IAT at post-TT10km confirms that core temperature is one of the underlying factors contributing to the diurnal variation in physical performance.

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Corresponding author: **Tarak Driss** Paris Nanterre University UFR STAPS, LINP2 200 avenue de la République 92001 Nanterre Cedex, France E-mail: tarak.driss@parisnanterre.fr

**ORCID:** Wajdi Souissi 0000-0003-1321-7906

Omar Hammouda 0000-0002-5002-687X

Achraf Ammar 0000-0003-0347-8053

Mohamed Ayachi 0000-0002-5261-3576

Tarak Driss 0000-0001-6109-7393

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#### INTRODUCTION

It is well established that several aspects of human functioning, such as physical performance, are time of day (TOD) dependent [1, 2]. Indeed, the diurnal variation of short-term performance has been widely confirmed by several previous studies with a nadir (minimum) in the morning and a peak (maximum) in the evening [1, 3, 4, 5, 6]. However, conflicting findings have been reported regarding the effect of TOD on long-duration endurance performance [1, 7, 8, 9]. Particularly, some previous investigations have shown better endurance performance in the evening compared to the morning during submaximal cycling [10] or maximal [11] running exercise. In contrast, findings from other studies failed to show significant diurnal variation of endurance performance during similar types of exercise [9, 12, 13].

The effect of TOD on cardiorespiratory responses also presented controversial findings during endurance exercises with some reports identifying a higher maximal oxygen uptake ( $\dot{V}O_2max$ ) [7, 11] and maximum heart rate ( $HR_{max}$ ) [7, 14, 15] in the evening hours, while other studies reported no diurnal variation of oxygen uptake ( $\dot{V}O_2$ ), heart rate (HR) or minute ventilation ( $\dot{V}E$ ) responses to similar endurance efforts [16, 17, 18]. These conflicting findings suggest that the extent of diurnal variation in endurance performance and cardiorespiratory responses remains uncertain.

Although athletes' pacing behaviour is widely recognized as an essential determinant of endurance performance, TOD effects have been examined considerably more during externally paced [1, 6, 7] than self-paced exercises [9, 19]. To the best of the authors' knowledge, only two studies have explored the diurnal variation of endurance performance and cardiorespiratory responses during self-paced cycling exercise [9, 19]. Findings from these studies were also

controversial, with one of them showing faster completion time and higher  $\dot{V}O_2$  in the evening compared to the morning during a 1 km cycling time trial (TT) [19], while the second one reported no diurnal variation of the same parameters during a 3 km TT [9].

These discrepancies could be related to methodological and individual issues, such as the small sample sizes, the habitual training time and the participant's chronotype [20] as well as training level [21]. In this line, Roveda et al. [8] revealed that participants reached their higher endurance performance at the same time as their habitual training sessions, which may mask the TOD effect.

All considered, it seems that the effect of TOD on endurance performance and cardiorespiratory function during self-paced exercise is still unclear and requires further studies. Therefore, the aim of the present study was to determine the diurnal variation of cycling performance, as well as cardiorespiratory and perceptual responses to a 10-km cycling time trial ( $TT_{10km}$ ) in well-trained team sport players. We hypothesized that physical, physiological and perceptual responses to  $TT_{10km}$  would be better in the evening than the morning hours.

#### MATERIALS AND METHODS

#### Participants

The required sample size was determined a priori, using the software G\*Power [22] and procedures suggested by Beck [23]. Values for power and  $\alpha$  were set at 0.95 and at 0.05, respectively. Based on the results of Knaier et al. [21] and Brisswalter et al. [24], effect sizes were estimated to be 0.45 (medium effect). To reach the desired power (0.9), data from at least twelve participants were deemed to be sufficient to minimize the risk of incurring a type 2 statistical error. Allowing for an 20% attrition rate, fifteen team sport players were recruited to participate in this study. To avoid possible confounding effect of training at a specific TOD [1, 25], three players, habituated to training only during the morning times, were excluded. The final sample included twelve physically trained subjects (mean  $\pm$  standard deviation (SD): 20.3  $\pm$  1.2 years, 74.3  $\pm$  7.4 kg, 179.7  $\pm$  5.5 cm, peak oxygen uptake ( $\dot{V}O_2$  peak) 58.3  $\pm$  7.7 ml. min<sup>-1</sup>.kg<sup>-1</sup>) volunteered to participate in this study. They trained four days per week for an average of three hours per day (90 min in the morning session and 90 min in the evening session). According to Horne and Östberg's chronotype questionnaire [26], all the participants were classified as intermediate type. They were non-smokers, without any risk factor of acute or chronic diseases (i.e., obesity, diabetes, cardiovascular or pulmonary disorders) and did not consume nutritional supplements, alcohol, or drugs during the experimental period and for at least one month before the commencement of the study. Participants signed an informed consent form to participate in the experiment after being informed about the experimental protocol and the potential risks and benefits of the study. The study design was approved by the local Institutional Review Board (approval code Nº 0183/2019) and carried out according to the guidelines of the Declaration of Helsinki for human experimentation.

### Experimental design

The experimental design was composed of four different sessions interspaced by 72 hours of rest in between. During the first session, participants were instructed and familiarised with the material and methods of the study before signing their informed consent. During the second session, participants performed familiarization with the TT<sub>10km</sub> to minimize bias and error related to learning effects. This familiarization session was performed between 12:00h and 13:00h in order to prevent adaptation to a particular experimental TOD. During this session, participants adjusted their seat position and chose the cycling braking load with which they achieved the best performance in the  $TT_{10km}$ . During the third and the fourth sessions, participants performed, in a randomised design, the  $\mathrm{TT}_{10\mathrm{km}}$  either in the morning (from 07:00h to 08:00h) or in the evening (from 17:00h to 18:00h). Intra-aural temperature (IAT) was assessed before and after the  $TT_{10km}$ . Power output (PO), rating of perceived exertion (RPE) and cardiorespiratory parameters (HR,  $\dot{V}E$ ,  $\dot{V}O_2$ , carbon dioxide production (VCO<sub>2</sub>) and respiratory exchange ratio (RER)) were averaged every covered km during the  $TT_{10km}$ . Completion time was also assessed during the TT<sub>10km</sub>. Blood lactate concentration [La] and glucose concentration [Glu] were assessed before and immediately following the completion of the first and the second 5 km of the  $TT_{10km}$ . Environmental temperature and relative humidity of the laboratory were maintained constant at 20 to 21°C and 40 to 45%, respectively. During the experimental sessions, participants maintained their habitual physical activity and were required to avoid intensive efforts for at least 24 hours before each experimental session.

# Endurance performance

The TT<sub>10km</sub> was conducted on a cycle ergometer (Monark 874E, Stockholm, Sweden) after a standardized warm-up at a constant power output of 100 W for 5 minutes. During the two experimental trials (morning and evening), using the same cycle ergometer and the same seat position, participants were instructed to complete the TT<sub>10km</sub> as fast as possible after the same warm-up and against the same cycling braking load (2.0 ± 0.2 kg) chosen in the familiarization trial. During TT<sub>10km</sub>, they were permitted to view elapsed distance, but they were blinded to power output and HR. No verbal encouragement was given during the two experimental trials. All data were averaged for each km before statistical analyses were performed.

# Cardiorespiratory variables

The cardiorespiratory parameters were assessed using a gas analyser (K4B2, Cosmed, Italy) connected to the heart rate monitor Polar H10 (Finland).  $\dot{V}E$ ,  $\dot{V}O_2$ ,  $\dot{V}CO_2$ , RER and HR were recorded and averaged for each covered km during the  $TT_{10km}$ .

# Blood lactate concentration

[La] was measured at rest, at 5 km and immediately after the  $TT_{10km}$ . A 5  $\mu$ l sample of whole fresh blood was taken from the fingertip and

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analysed for lactate concentration (mmol/l) using a portable analyser (lactate Pro2, Arkray, Shiga, Japan).

#### Blood glucose concentration

[Glu] was measured at rest, at 5 km and immediately after  $TT_{10km}$ . A 5  $\mu$ l sample of whole fresh blood was taken from the fingertip and analysed for blood glucose concentration (g/l) using a portable analyser (Contour Next, USA).

# Intra-aural temperature

Intra-aural temperature (IAT) was measured using a digital infrared thermometer (Braun, ThermoScan 6, France) before and immediately after the TT<sub>10km</sub>.

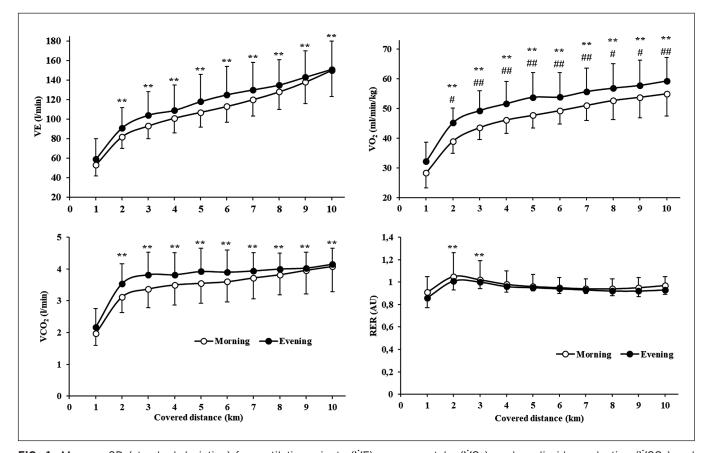
# Rating of perceived exertion

The RPE scale is a reliable indicator of physical discomfort, has sound psychometric properties, and is strongly correlated with several other physiological measures of exertion [27]. In this study, we used a 10-point scale ranging from 1 (very very light) to 10 (very very

hard). Every 1 km of  $\text{TT}_{10\text{km}}$ , participants were requested to rate their subjective exertion.

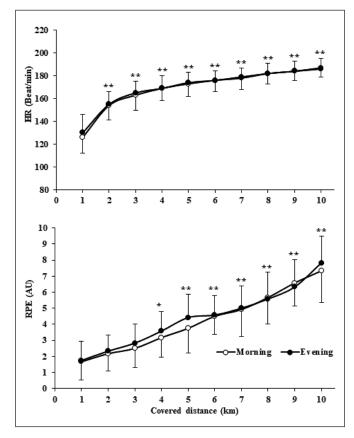
# Statistical analysis

Statistical analyses were performed using STATISTICA software (Stat-Soft, Paris, France). All data were presented as mean  $\pm$  standard deviation (SD). Once the assumption of normality was confirmed using the Shapiro-Wilk W test, parametric tests were performed. Two-way repeated measure ANOVA [2 TOD (morning vs. evening)  $\times$  10 covered km] was used to compare morning-evening differences for PO, cardiorespiratory parameters (HR, VE, VO<sub>2</sub>, VCO<sub>2</sub>, RER) and RPE. Moreover, repeated measure ANOVA [2 TOD (morning vs. evening)  $\times$  3 covered distance (at 0, 5 and 10 km)] was performed to compare [La] and [Glu] concentration differences. In addition, repeated measure ANOVA [2 conditions (morning vs. evening)  $\times$  2 covered km (at 0 and 10 km)] was conducted to compare IAT differences. When significant main or interaction effects were observed, the Bonferroni post-hoc test was conducted. Effect size was calculated as partial eta squared ( $\eta_0^2$ ). The paired samples t-test



**FIG. 1.** Mean  $\pm$  SD (standard deviation) for ventilation minute (VE), oxygen uptake (VO<sub>2</sub>), carbon dioxide production (VCO<sub>2</sub>) and respiratory exchange ratio (RER) during the 10 km cycling time trial in the morning and in the evening. # significant effect of time of day (TOD) (p < 0.05), ## significant effect of time of day (TOD) (p < 0.0005), \*Significantly different than 1 km value for the same time of day (p < 0.05) and \*\* significantly different than the 1 km value for the same time of day

(p < 0.001)



**FIG. 2.** Mean  $\pm$  SD (standard deviation) for heart rate (HR) and rating of perceived exertion (RPE) during the 10 km cycling time trial in the morning and in the evening.

\*Significantly different than 1 km value for the same time of day (p < 0.05) and \*\* significantly different than the 1 km value for the same time of day (p < 0.001).

was used to determine differences of the completion time of  $TT_{10km}$ . The effect size was calculated using Cohen's corrected (d) test [28] and the magnitude of changes using the following criteria: < 0.2 = trivial, 0.2-0.6 = small, 0.6-1.2 = moderate, 1.2-2.0 = large, and 2.0 = very large. Pearson correlation was conducted to examine the relationships between parametric variables. When the STATISTICA output demonstrated significance levels of p = 0.000, these were corrected to p < 0.0005 [29]. The significance level for all statistical procedures was set at p < 0.05.

# RESULTS

# Endurance performance

The completion time of the TT<sub>10km</sub> was shorter in the evening compared to the morning [(804 ± 89 s vs. 823 ± 78 s; t = 2.35, p = 0.04, d = 0.66]. Elapsed time was higher in the morning compared to the evening [F<sub>(9,99)</sub> = 172.9, p < 0.0005,  $\eta_p^2 = 0.92$ ].

Mean PO values in each 1 km of the  $TT_{10km}$  were not different between morning and evening [F<sub>(1,11)</sub> = 2.19, p = 0.16].

# Cardiorespiratory responses

Mean (± SD) values of  $\dot{V}$ E,  $\dot{V}O_2$ ,  $\dot{V}CO_2$  and RER in each 1 km of the TT<sub>10 km</sub> are displayed in Figure 1.

Repeated measure ANOVA revealed a significant main effect of covered km on HR [F<sub>(9,99)</sub> = 182.24, p < 0.0005,  $\eta_p^2$  = 0.94] (Figure 2),  $\dot{V}E$  [F<sub>(9,99)</sub> = 125.58, p < 0.0005,  $\eta_p^2$  = 0.92],  $\dot{V}O_2$  [F<sub>(9,99)</sub> = 84.79, p < 0.0005,  $\eta_p^2$  = 0.89],  $\dot{V}CO_2$  [F<sub>(9,99)</sub> = 48.64, p < 0.0005,  $\eta_p^2$  = 0.82] and RER [F<sub>(9,99)</sub> = 14.03, p < 0.0005,  $\eta_p^2$  = 0.56] values. At both TOD, all these cardiorespiratory responses (except RER) increased continuously through the 10<sup>th</sup> covered km (p < 0.001). A significant increase in RER [F<sub>(9,99)</sub> = 14.03, p = 0.000,  $\eta_p^2$  = 0.56] was only observed at 2 km and 3 km compared to 1 km in both conditions. A significant main effect of TOD was observed only for  $\dot{V}O_2$  [F<sub>(1,11)</sub> = 8.27, p = 0.02,  $\eta_p^2$  = 0.43], with higher  $\dot{V}O_2$  values observed in the evening compared to the morning (0.0005 < p < 0.04).

### Blood lactate concentration

Repeated measure ANOVA showed a significant effect of  $TT_{10km}$  [F<sub>(2,22)</sub> = 192.01, p < 0.0005,  $\eta_p^2$  = 0.94] and TOD [F<sub>(1,11)</sub> = 5.75, p = 0.04,  $\eta_p^2$  = 0.34] on [La] with a rising level from rest to 5 km and 10 km (p < 0.0005) and from 5 km to 10 km (p < 0.0005) in both morning and evening conditions (Table 1). Moreover, at 10 km [La] showed higher values in the evening compared to the morning at 10 km, affected by TOD (p = 0.04).

#### Glucose concentration

Repeated measure ANOVA showed a significant effect of  $TT_{10km}$  [ $F_{(2,22)} = 66.72$ , p < 0.0005,  $\eta_p^2 = 0.86$ ] and TOD [ $F_{(1,11)} = 8.58$ , p = 0.014,  $\eta_p^2 = 0.44$ ] on [Glu] with a rising level from rest to 5 km (p = 0.01 in the morning and p < 0.0005 in the evening) and 10 km (p < 0.0005) and from 5 km to 10 km (p < 0.0005) in both morning and evening conditions (Table 1). Moreover, [Glu] was higher in the evening compared to the morning at 5 km (p = 0.03) and 10 km (p = 0.001).

# Intra-aural temperature

Repeated measure ANOVA revealed a significant main effect of TT<sub>10km</sub> [F<sub>(1,11)</sub> = 33.30, p < 0.0005,  $\eta_p^2$  = 0.75] and TOD [F<sub>(1,11)</sub> = 7.20, p = 0.02,  $\eta_p^2$  = 0.40] on IAT. Compared to the rest values, IAT increased significantly following TT<sub>10 km</sub> in the evening (36.89 ± 0.39 vs. 37.33 ± 0.43, p = 0.010) and in the morning (36.58 ± 0.22°C vs. 37.11 ± 0.34°C, p = 0.002), with higher pre-TT<sub>10km</sub> values registered during the evening compared to the morning session (p = 0.02,  $\Delta$  = 0.33°C). In addition, a high negative correlation was found between  $\Delta$  completion time (morning to evening) and  $\Delta$  IAT (morning to evening) at post-TT<sub>10km</sub> (r = -0.83, p = 0.04).

# Rating of perceived exertion

Mean ( $\pm$  SD) RPE scores are presented in Figure 2. In both TOD, RPE scores significantly increased at each successive 1 km of the

 $TT_{10 \text{ km}} [F_{(9,99)} = 65.77, p < 0.0005, \eta_{p}^{2} = 0.86]$ , with no significant effect of TOD.

#### DISCUSSION

The main purpose of the present study was to determine the effect of TOD on physical, physiological and perceptual responses to the  $TT_{10km}$ . The primary findings indicated better endurance performance with higher IAT,  $\dot{V}O_2$ , [La] and [Glu] values during the  $TT_{10km}$  performed in the evening compared to the morning. However,  $\dot{V}E$ ,  $\dot{V}CO_2$ , RER and RPE values were unchanged between conditions.

The present study reported a significant TOD effect on TT<sub>10km</sub> performance with faster completion time in the evening compared to the morning. These findings are in line with previous studies showing a greater endurance performance in the evening during a cycling time trial [10, 14, 19, 30], intermittent running test [7] or self-paced running exercise [8]. However, these findings are in contrast with other studies demonstrating no diurnal variation of endurance performance in response to a self-paced cycling time trial: 4 km [9], 15 min [31], 4×5-min [17], submaximal cycling exercise [12], maximal cycling [32] or running [33] exercises. These discrepancies could be explained by the chronotype of the participants and/or the habitual training time [20, 21, 34]. Indeed, Knaier et al. [21] suggested that participants reached their peak endurance performance at the same time as their habitual training sessions, which may mask the TOD effect. In the same way, a recent study by Roveda et al. [8] indicated that participants' chronotype has the potential to influence the TOD when peak performance is achieved and showed that the morning-type group tends to perform better in the morning while the evening-type group tends to perform better in the evening. These results suggest that recruiting a specific number of morning-type participants may mask the diurnal variation of endurance performance and thereby explain the absence of a TOD effect in some previous studies. The findings of the present study confirm these recent suggestions and showed that, using team sport players of intermediate chronotype and with no specific habitual training time, a significant TOD effect on endurance performance was registered with faster completion time during the  $TT_{10km}$  observed in the evening.

This better physical performance registered during the  $TT_{10km}$  performed from 17:00h to 18:00h was associated with higher IAT at post- $TT_{10km}$ , which suggests the possibility of a causal effect between changes in IAT and fluctuations in endurance performance. Particularly, the results of the present study showed a high negative correlation (r = -0.76) between the diurnal fluctuation of completion time and IAT at post- $TT_{10km}$ . This finding may indicate that morning-evening increase in IAT (and consequently core temperature) could explain in part the decreased completion time and thereby the better physical performance recorded in the evening. In line with this speculation and given the similar magnitude of the TOD effect on IAT and physical performance, changes in core temperature have been frequently used to explain the enhanced performance in the late after-

noon and evening [3, 4, 5, 21, 35]. Indeed, it has been previously suggested that the increase in core temperature during the day can serve as a passive muscle warm-up improving the efficiency of the neuromuscular system through (i) increasing nerve conduction velocity, body chemical reaction, muscle vascularization and range of motion and (ii) decreasing muscle viscosity [14, 36, 37, 38, 39]. However, Atkinson et al. [30] stated that diurnal variation of core temperature cannot solely explain the TOD differences in performance and suggested that explaining the underlying physiological mechanism of diurnal variation in physical performance needs further studies measuring a broad range of possible psychophysiological responses accompanying the completion of exercise at different TOD.

In this context, aiming to better understand the underlying mechanism behind the endurance performance fluctuation, the present study also examined the diurnal variation of cardiorespiratory, metabolic ([La] and [Glu]) and perceptual responses to  $TT_{10km}$  performed at two different TOD. VO2, [La] and [Glu] were found to be significantly affected by the TOD. The present findings are in accordance with other studies showing a higher [La] and  $\dot{V}O_2$  in the evening compared to the morning during a 1000 m cycling time trial [19], endurance exercise above the ventilatory threshold [38, 42] or during the YoYo intermittent recovery test [7, 11]. However, the present results are in contrast with several previous studies that failed to provide evidence for significant diurnal variation of VO<sub>2</sub> and/or [La] during submaximal [12, 18, 40], maximal [7, 21, 32, 41] or selfpaced [9, 19, 35] exercises. These results could be explained by an achieved VO<sub>2max</sub>, [La] and RPE at different TOD by different athletes [21]. Discrepancies between findings suggest that the protocol of the used endurance exercise seems to be also one of the methodological factors influencing the TOD effect when peak psychophysiological responses are achieved. Additionally, the intermediate chronotype in all the recruited subjects in the present study could also explain, in part, the absence of diurnal variation in physiological (HR,  $\dot{V}E$ ,  $\dot{V}CO_2$ , RER) and perceptual (RPE) responses to the  $TT_{10km}$ . Therefore, it is necessary to conduct further TOD studies controlling as many influencing factors as possible (i.e., chronotype, habitual time of training, training level, exercise protocol) and testing at the same time a wide range of psychological (e.g., motivation, psychomotor) and physiological (hormonal profile, inflammatory and muscle damage) responses.

#### CONCLUSIONS

According to the present findings, the evening session seems to result in better endurance performance, which was accompanied by higher IAT,  $\dot{V}O_2$ , [La] and [Glu] compared to the morning session. The similar magnitude of TOD effect on IAT,  $\dot{V}O_2$ , [La], [Glu] and  $TT_{10km}$ completion time and the absence of diurnal variation in the remaining physiological (i.e., HR,  $\dot{V}CO_2$ , RER,  $\dot{V}E$ ) and perceptual (RPE) parameters confirm that core temperature, oxygen consumption and metabolic responses are important underlying factors contributing to the diurnal variation in endurance cycling performance. It is recommended for athletes, coaches and sport scientists to consider self-paced endurance performance as time-of-day dependent. Importantly, endurance performance during the 10 km cycling time trial exercise should be scheduled in the evening to achieve better performance. However, the TOD of the competition should also be considered when scheduling the training programme to benefit from specific adaptations at this TOD. Indeed, a previous report indicated that adaptations to training are greater at the TOD at which training is regularly performed than at other times [1]. Finally, to guarantee comparable results in future TOD-based investigations, it is recommended to recruit participants with an intermediate chronotype when assessing metabolic responses (i.e., oxygen uptake, and blood glucose and lactate levels) to physical exercise.

# Limitations

In the present study, a small sample size of participants with inter-

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mediate chronotype was included in the experimental procedure. Moreover, endurance performance was measured only at two times of day. More studies are required to investigate the effect of different times of day on endurance using different test times and different participants' chronotypes.

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